

Coupled nucleoside kinase - IMPDH II assay

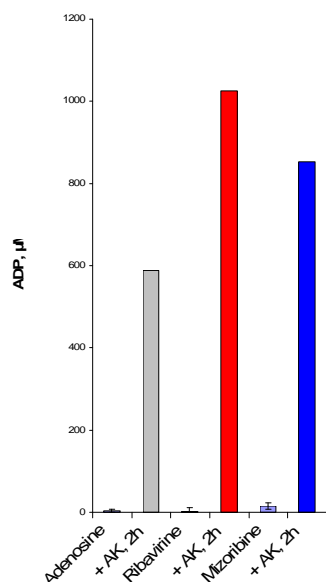
IMPORTANT: Client-specified alterations can be accommodated.

IMP Dehydrogenase (IMPDH, E.C. 1.1.1.205) catalyzes the pivotal step in guanine nucleotide biosynthesis. By converting inosine monophosphate (IMP) to xanthosine monophosphate (XMP), IMPDH controls the guanine nucleotide pool. A number of nucleoside analogues (e.g. **ribavirin**, **mizoribine**) are known to inhibit IMPDH after being monophosphorylated. The therapeutic consequences of IMPDH inhibition vary from different analogues - mizoribine is an immunosuppressor and ribavirin is a broad spectrum antiviral. Even if direct relationship between ribavirin antiviral action and IMPDH inhibition by ribavirin monophosphate has not been demonstrated, the depletion of cellular GTP might result in an increased frequency of ribavirin triphosphate incorporation by viral polymerase due to a lower intracellular concentration of its natural competitor.

Aim: For rapid evaluation of monophosphate forms of nucleoside analogues as IMPDH inhibitors.

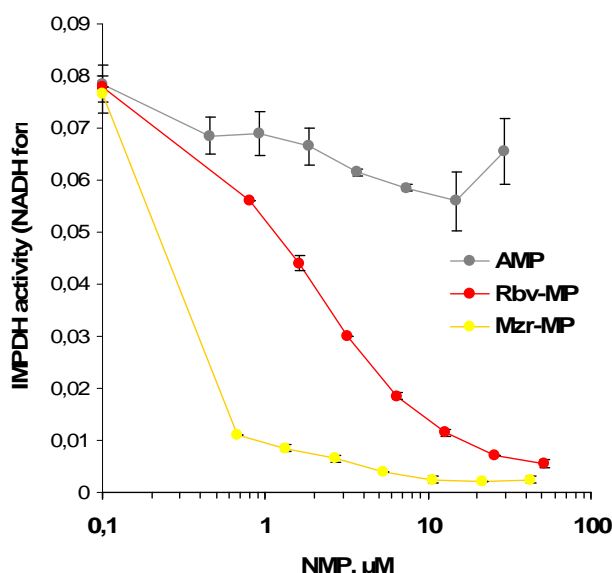
	AK	5'cN-II
Natural substrates	Adenosine Inosine	Deoxyinosine Inosine
Nucleoside analogues substrates	Ribavirin Tubercidin Mizoribine	Dideoxyinosine Ribavirin Acyclovir

Enzymes: The monophosphorylation step of nucleoside analogue is provided by one of the specific human recombinant nucleoside kinases: AK (ref. # E-Nov 5) or cN-II (ref. # E-Nov 6) produced by **NOVO CIB**. Human recombinant IMPDH 2 was cloned from human cells, expressed in *E. coli* and purified by **NOVO CIB** (see sheet # E-Nov 1 for further information). The enzyme purity is controlled by SDS-PAGE, protein concentration is measured by Bradford method (Bio-Rad). A standard operating procedure (SOP) is followed to measure enzymatic activity.



Phosphorylation of nucleoside analogue is provided by specific nucleoside kinase and confirmed by spectrophotometric quantification of formed ADP in LDH-PK coupled reaction.

A confirmation by HPLC analysis of formation of monophosphorylated forms is available upon request.



IMPDH inhibition: Effect of monophosphorylated nucleoside analogues on human recombinant IMPDH II. Enzymatic assays performed in duplicate are carried out at 37°C in 0.1M KH₂PO₄ buffer pH 8.0 in the presence of 2mMDTT, 200µM NAD, 200µM IMP and 0.2 µM IMPDH II and increasing concentration of monophosphorylated nucleoside. Reaction is followed in an iEMS Reader MF (Labsystems) microtiter plate reader at 340nm.

References

- 1] P. Leyssen, J. Balzarini, E. De Clercq, J. Neyts (2005) The Predominant Mechanism by Which Ribavirin Exerts Its Antiviral Activity In Vitro against Flaviviruses and Paramyxoviruses Is Mediated by Inhibition of IMP Dehydrogenase J Virol 79: 1943–1947
- [2] L.J. Stuyver, S. Lostia, S.E. Patterson, J.L. Clark, K. A. Watanabe, M.J. Otto and K.W. Pankiewicz (2002) Inhibitors of the IMPDH enzyme as potential antiviral agents Antiviral Chemistry & Chemotherapy 13:345–352

Related products:

- **dCK nucleoside phosphorylation assay**
- **Adenosine kinase nucleoside phosphorylation assays**
- **cN-II phosphorylation assay**
- **Deoxycytidine kinase (dCK)**
- **Adenosine kinase**
- **Cytosolic 5' nucleotidase II (cN-II)**
- **UMP-CMP kinase (CMK)**
- **CMK nucleotide monophosphate phosphorylation assay**
- **Coupled dCK-CMK nucleoside phosphorylation assays**